

AMENDMENTS TO THE CLAIMS

This Listing of Claims will replace all prior versions, and listings, of claims in this application.

Listing of Claims:

Claim 1 (**currently amended**): A combinatorial protein library comprising a plurality of protein species, each protein species comprising an A chain of an ABx toxic protein ~~into which an insert has been introduced,~~

wherein the A chain of the ABx toxic protein comprises a protease-sensitive loop or region into which ~~the~~ an insert has been introduced, and

wherein the insert of each protein species is a polypeptide of varying amino acid sequence having a length of at least 2 amino acid residues, and

wherein the library comprises at least two different protein species.

Claim 2 (**previously presented**): The combinatorial protein library of claim 1, wherein the library comprises at least 100 protein species.

Claim 3 (**previously presented**): The combinatorial protein library of claim 1, wherein the protein species are formed by introducing the insert into a Shiga-like toxin I A chain.

Claim 4 (**currently amended**): The combinatorial protein library of claim 3, wherein ~~the~~ one or more protein species comprise

a) A1 domain sequences sufficient to retain A1 domain catalytic activity selected from amino acid residues 7 to 257 of SEQ ID NO.: 1, and

b) a protease-sensitive sequence C-terminal to said A1 domain sequences into which an insert may be introduced without abolishing catalytic activity of A1 domain sequences sufficient to retain catalytic activity and A2 domain sequences such that the insert may be introduced between amino acids 248 and 267, as defined with reference to SEQ ID No.:
1.

Claim 5 (**currently amended**): The combinatorial protein library of claim 4, wherein the ~~protein species is formed by introducing the insert~~ is introduced between amino acids 251 and 252, ~~as defined with reference to~~ of SEQ ID NO~~e~~.: 1.

Claim 6 (**canceled**).

Claim 7 (**previously presented**): The combinatorial protein library of claim 1, wherein the insert has a length of 7 amino acids.

Claim 8 (**withdrawn, currently amended**): A combinatorial expression library comprising a plurality of members, wherein each member comprises a DNA sequence encoding a protein species, each protein species comprising an A chain of an ABx toxic protein ~~into which an insert has been introduced,~~

wherein the A chain of the ABx toxic protein comprises a protease-sensitive loop or region into which ~~the~~ an insert has been introduced, and

wherein the insert of each protein species ~~encodes~~ a polypeptide of varying amino acid sequence having a length of at least 2 amino acid residues, and

wherein the library comprises at least two different members.

Claim 9 (**withdrawn, currently amended**): A mutant protein comprising an A chain of an ABx toxic protein ~~into which an insert has been introduced,~~

wherein the A chain of the ABx toxic protein comprises a protease-sensitive loop or region into which ~~the~~an insert has been introduced, and

wherein the insert is a polypeptide of varying amino acid sequence having a length of at least 2 amino acid residues.

Claim 10 (**withdrawn**): The mutant protein of claim 9, wherein the A chain of a toxic protein is a Shiga-like toxin I A chain.

Claim 11 (**withdrawn, currently amended**): The mutant protein of claim 10, wherein the Shiga-like toxin I A chain sequence is selected from SEQ ID NO.: 1, and wherein the insert is introduced between amino acids 248 and 267,~~as defined with reference to~~ of SEQ ID NO.: 1.

Claim 12 (**withdrawn, currently amended**): The mutant protein of claim 11, wherein the insert is introduced between amino acids 251 and 252,~~as defined with reference to~~ of SEQ ID NO.: 1.

Claim 13 (**withdrawn, currently amended**): The mutant protein of claim 12, wherein the insert comprises the sequence IYSNKLM (SEQ ID NO.: 6).

Claim 14 (**withdrawn, currently amended**): The mutant protein of claim 12, wherein the insert comprises the sequence AAFADLI (SEQ ID NO.: 7).

Claim 15 (**withdrawn, currently amended**): The mutant protein of claim 10, wherein the insert is introduced before or after amino acids 1-239 of the Shiga-like toxin I A chain, as defined with reference to SEQ ID NO.: 1.

Claim 16 (**withdrawn**): The mutant protein of claim 9, wherein the insert has a length of 7 amino acids.

Claim 17 (**withdrawn**): A method for identifying a ligand that binds to a specific cell surface target/receptor, comprising the steps of:

(a) exposing cells to one or more members of a combinatorial protein library in accordance with claim 1;

(b) selecting members of the protein library that are observed to be toxic to the cells upon cell binding or internalization;

(c) evaluating the selected members of the protein library to determine the sequence of the inserted region, wherein the inserted sequence is determined by sequencing the amino acids or the nucleic acids encoding the amino acids of the inserted sequence; and

(d) identifying a peptide of the sequence of the inserted region as a possible ligand for a target/receptor on the exposed cell.

Claim 18 (**withdrawn**): A method for isolating a toxin specific for a target/receptor comprising the steps of:

(a) exposing the target/receptor to a combinatorial protein library according to claim 1; and

(b) isolating at least one protein species from the combinatorial protein library captured by binding to the target/receptor.

Claim 19 (**withdrawn**): The method of claim 18, further comprising the step of screening the isolated protein against cells expressing the target/receptor, to confirm their toxicity for cells expressing the target/receptor.

Claim 20 (**withdrawn**): The method of claim 18, wherein the target/receptor is a purified target/receptor and is immobilized on a solid support.

Claim 21 (**withdrawn**): The method of claim 20, wherein the target/receptor is on the surface of cells.

Claim 22 (**withdrawn**): The method of claim 21, wherein the cells are immobilized on a solid support.

Claim 23 (**withdrawn**). The method of claim 21, wherein the toxin serves as a reporter, and the death of the cells is indicative of receptor binding.

Claim 24 (**withdrawn**): The combinatorial expression library of claim 8, wherein the DNA sequence is introduced into an expression vector.

Claim 25 (**withdrawn**): The combinatorial expression library of claim 24, wherein the expression vector is of bacterial, viral or yeast origin.

Claim 26 (**withdrawn**): The combinatorial expression library of claim 8, wherein the expression vector is a plasmid.

Claim 27 (**withdrawn**): The combinatorial expression library of claim 8, wherein the expression vector is a viral vector.

Claim 28 (**withdrawn**): The combinatorial expression library of claim 8, wherein the library comprises at least 100 members.

Claim 29 (**withdrawn**): The combinatorial expression library of claim 8, wherein the protein species encoded by the DNA members are formed by introducing the insert into a Shiga-like toxin I A chain.

Claim 30 (**withdrawn, currently amended**): The combinatorial expression library of claim 29, wherein the protein species encoded by the DNA members comprise sequences selected from SEQ ID NO.: 1, and wherein said species are formed by introducing the insert between amino acids 248 and 267,~~as defined with reference to~~ of SEQ ID NO.: 1.

Claim 31 (**withdrawn, currently amended**): The combinatorial expression library of claim 30, wherein the protein species encoded by the DNA members are formed by introducing the insert between amino acids 251 and 252, ~~as defined with reference to~~ of SEQ ID NO.: 1.

Claim 32 (**withdrawn**): The combinatorial expression library of claim 29, wherein the protein species is formed by introducing the insert before or after amino acids 1-239 of the Shiga-like toxin I A chain, as defined with reference to SEQ ID NO.: 1.

Claim 33 (**withdrawn**): The combinatorial expression library of claim 8, wherein the insert has a length of 7 amino acids.

Claim 34 (**withdrawn**): The method of claim 17, further comprising the step of testing the peptide of the sequence of the inserted region to confirm that it is a ligand for the specific cell surface target/receptor.

Claim 35 (**withdrawn**): The method of claim 17, wherein the specific cell surface target/receptor is known to be present on one or more of the exposed cells.

Claim 36 (**new**): The combinatorial protein library of claim 4, wherein the insert is introduced between amino acids 248 and 267 of SEQ ID NO.: 1.